# RED MUD AS A CONSTRUCTION MATERIAL BY USING BIOREMEDIATION

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By


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**C E R T I F I C A T E**

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This is to certify that the thesis entitled, "**Red mud as a construction material by using bioremediation**" is submitted by surabhi jain, Roll No. 212CE1477 in partial fulfilment of the requirements for the award of Master of Technology degree in Civil Engineering with specialization in "Geotechnical Engineering" during 2012-2014 session at the National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Dr. Sarat Kumar Das

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# Abstract

Rapid industrialization and faster growth rate are the requirements for leading a proficient life but a holistic approach with environmental consideration are essential for sustainable development. These industries are partially fulfilling their tasks since many factors are not overcome by them successfully and one of that is safe disposal of waste generating at the end.

Red mud is a waste product from the Alumina industry and it creates a lot of health hazards to the ecology, if it is left disposed without necessary precautions, hence safe disposal practices and reuse of the product are one of the solutions. Disposal method entails a huge land area and enormous mass of earth material for construction of embankment. Many countries are disposing red mud waste directly into the ocean due to shortage of land area and scarcity of earth material.

Many researches are still being carried out on the neutralisation of red mud in various ways. This report is one of the parts of utilizing the red mud in a very better and economic manner. In this paper the red mud is used as an alternative construction material after remediation by biological process. This gives a cost effective neutralisation method as well as abundant material which can use in construction.

Research in biology and earth science has enabled important advances in understanding the crucial involvement of microorganisms in the evolution of the earth, their ubiquitous presence in near surface soils and rocks, and their participation in mediating and facilitating most geochemical reactions. Yet, the effect of biological activity on soil mechanical behaviour remains largely unexplored in the Geotechnical field. This research Provides examples of how microbiological conditions and processes may influence engineering

properties and behaviours of earth materials which opens a new biological field in Geotechnical engineering which is known as **Bio-Geotechnics**.

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# CHAPTER 1

# INTRODUCTION

## 1.1 INTRODUCTION

The requirements for leading a proficient life is challenging for the rapid development of industries and they partially fulfil their tasks since many factors are not overcome by them successfully and one of that is safe disposal and utilisation of waste generating at the end.

The waste of aluminium industry known as red mud or bauxite residue is discharged when alumina is coming out from bauxite. During the most feasible Bayer process alumina is extracted from bauxite at elevated temperature and pressure with the presence of sodium hydroxide. Red mud generation is depending upon the type of bauxite used in industry. About 1.2-1.4 tons of red mud is generated per each ton of alumina produced. Each year, more or less 75 million tonnes of red mud is produced worldwide. The iron compounds present in it confers the red colour to it and hence it is called red mud.

The problem with the red mud is that it is toxic by nature. The chemical analysis conducted on red mud reveal that it contains silica, aluminium, iron, calcium, titanium, as well as an array of minor constituents, namely: Na, K, Cr, V, Ni, Ba, Cu, Mn, Pb, Zn etc, because of the harmful chemical composition present in it but the major problem of red mud is it is caustic in nature as the alkalinity is very high. The pH value of red mud is varies from 10.5 to 13. This waste is usually managed by discharge into engineered or natural impoundment reservoirs, with subsequent dewatering by gravity-driven consolidation and sometimes followed by capping for closure. Due to the alkaline nature it neither is used for construction material nor for vegetation.

The environmental trouble linked with the disposal of red mud waste includes:

- The high pH (10.00-13.00).
- Contamination of underground water due to alkali seepage.
- Storage of red mud is not stable.
- Alkaline air effect to plant life.
- Enormous areas of land consumed.

Figure 1.1 Discharge of Red Mud as Slurry into thePond

## 1.2 DEFINITION OF PROBLEM

Various researches are going on to utilise the bauxite residue but only 5% is used so different efforts are going on to neutralise the red mud. **Neutralization** of red mud will help to lessen the environmental impact caused due to its storage and also lessen significantly the ongoing management of the deposits after closure. It will also open opportunities for re-use of the residue which to date have been prevented because of the high pH. The cost of neutralization will, to some degree at least, be equalizing by a reduction in the need for long-term management of the residue deposits. The funds can be used for neutralisation rather than using for storage of red mud.

The processes already used for neutralisation are sea water neutralisation, acid waste water neutralisation, carbon dioxide neutralisation. The turbidly of sea is increased and marine environment get effected due to the sea water neutralisation. Various new as carbonate and other reagents are coming out due to waste water neutralisation and cannot satisfy the industrial demand. The carbon dioxide neutralisation is not feasible as red mud cannot be neutralising up to the mark.

**Bioremediation** is a technology that utilizes the biological activity to reduce or eliminate environmental hazards resulting from the accumulation of toxic chemicals and other hazardous wastes. It is a versatile process because it can be adapted to suit the specific needs of each site. This process is still considered an innovative technology that has been used in a limited number of cases with several advantages in spite of certain disadvantages.

## 1.3 SCOPE AND OBJECTIVE OF RESEARCH WORK

The disposal of red mud is a threat to the environment due to its high alkalinity. Researchers are trying to use and neutralize it by physical and chemical method but the limitations faced with physical and chemical treatment technologies will be overcome with the help of microbes.

The purpose of this research work will be utilisation of the red mud, an industrial waste in the construction of embankment and fill which can't be done due to its high alkalinity. A new concept will be used to neutralize the red mud by biological activities so that the modified red mud can be tested in the field and advantageous for construction material which will reduce the need of borrowed soil.

**Biogeotechnics** is a new frontier in civil engineering where living organisms are used to bring desired chemical and physical change in a confined and regulated soil environment so these can be used in Geotechnical engineering. In this project the alkalinity of red mud will be trying to reduce by micro-organisms with different novel concepts. All the morphology and Geotechnical properties as well as chemical characteristics of red mud will be identified. A good comparison is done before and after bioremediation.

## 1.4 THESIS OUTLINE

A brief description of red mud and neutralisation of red mud is described in chapter 1 as Introduction. Different bioremediation procedure and geotechnical experiments which have been done is presented in chapter 2 as literature review.

The different materials used in the project work are described in chapter 3. The methods applied for bio-neutralisation, chemical and geotechnical properties of samples are given in this chapter.

Chapter 4presentresults coming out from the methods used for bio-neutralisation, chemical properties of red mud and properties of red mud and bio-neutralised red mud. It discusses about the comparison of both red mud and neutralise red mud.In chapter 5 conclusions drawn from various studies made in this thesis are presented and the scope for the future work is indicated. The general layout of the thesis work based on each chapter is shown in a flow diagram (Figure 1.2).

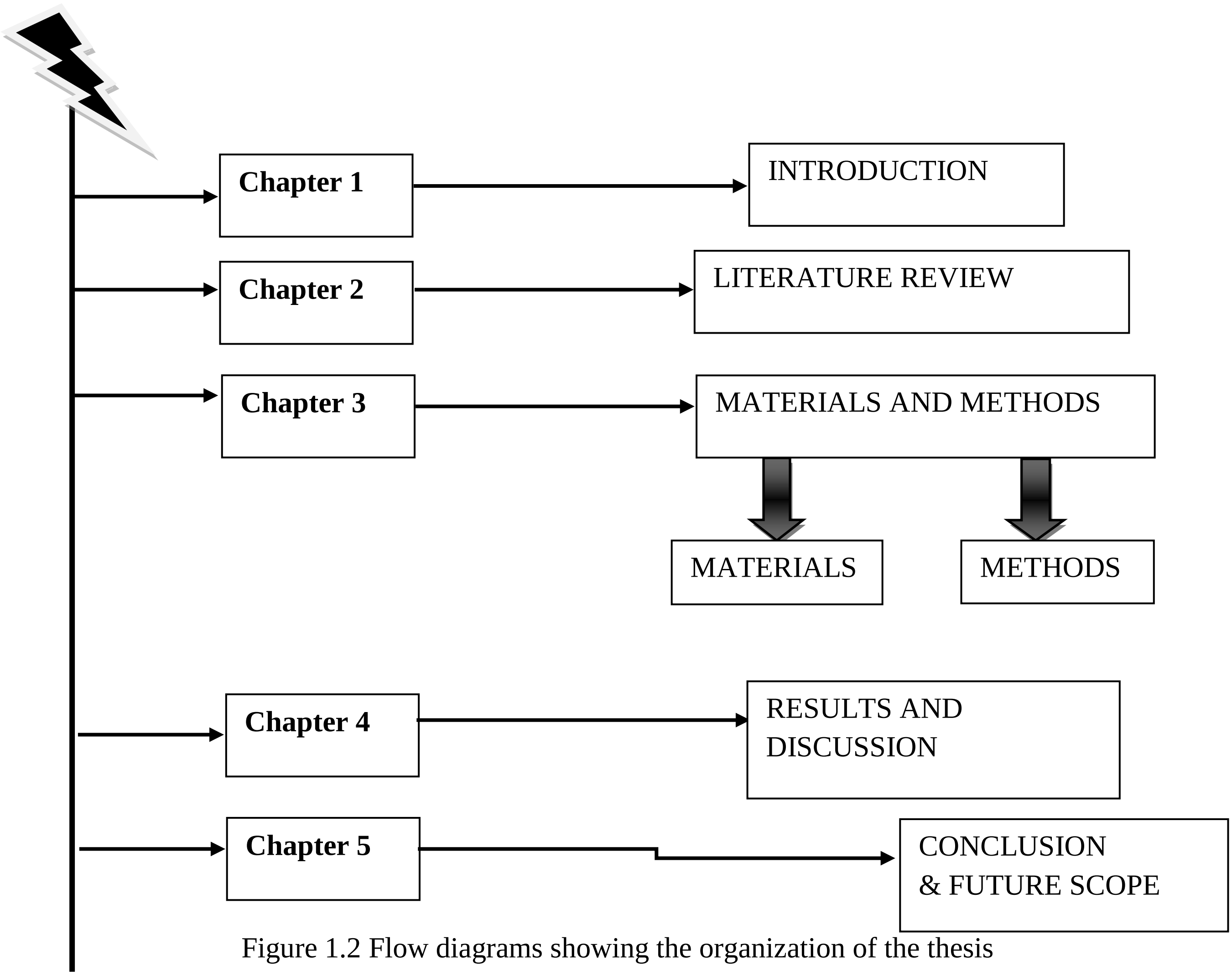


Figure 1.2 Flow diagrams showing the organization of the thesis

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1 INTRODUCTION

Though, various efforts have been made for geotechnical characterization of red mud, but no attempts have been made in geotechnical characterization of modified/stabilized red mud. But in other branches of engineering and science, few efforts have been made towards the Bio-neutralisation of red mud for reducing the alkalinity of red mud. As discussed in previous chapter, Bioremediation is a technology that utilizes the biological activity to reduce or eliminate environmental hazards resulting from the accumulation of toxic chemicals and other hazardous wastes. This chapter discusses about the different investigation for effective bioremediation of red mud in different applications in general. Thereafter, specific literature pertains to bioremediation application is presented. The literature pertaining to geotechnical investigation on red mud is also presented at the end.

## 2.2 DIFFERENT BIOREMEDIATION STUDY ON RED MUD

Mussels et al (1993) did a review to assess the feasibility of bioremediation of bauxite residue by making estimates of the possible substrate and inoculums required to achieve neutrality. Various possible avenues of microbial neutralization were assessed in terms of the practicality of application of bauxite residue. These involved the ability of an organism to survive and grow in red mud and their production of neutralizing agent mainly organic acid and carbon dioxide.

Hamdy and Williams (2001) demonstrated that low levels of injured bacterial cells in the bauxite residue actively grew used various added nutrients and/or hay. The organisms grew from less than 10 to more than $10^9$ cells $g^{-1}$ bauxite residue and formed organic acids that lowered the pH from 13 to about 7.0. A total of 150 cultures was isolated from treated bauxite residue and included species of Bacillus, Lactobacillus, Leuconostoc, Micrococcus, Staphylococcus, Pseudomonas, Flavobacterium and Enterobacter. Scanning electron micrographs demonstrated that untreated particles (control) of the bauxite residue were clumped together, and in treating bauxite residue these particles were highly dispersed with micro colonial structures. Furthermore, the treated bauxite residue supported the growth of several plants and earthworms that survived for over 300 days.

Krishna et al. (2004) gave a report on bioremediation of red mud by using fungus (Aspergillustubingensis). Aspergillus tubingensis was tested for its ability to grow at different pH, red mud amended media and tolerance to Na, Al and Fe. Different concentrations of red mud (0, 1, 2, 5 and 10%) were amended in Czepeck liquid media and the pH was recorded. The growth of A. Tubingensis was significantly inhibited by Al at 200µg/ml; however at 50µg/ml of Al, the growth was increased when compared to the control. To determine the maximum concentration of NaCl tolerated by A. tubingensis, different concentrations of Na were amended and the results showed that up to 50µg/ml of Na, the growth of A.tubingensis was not affected and at higher concentrations (150 and 200 µg/ml) the growth was significantly inhibited. The new content increased in the mycelium as the concentration of Na increases in the growth medium and the maximum accumulation was found at 200 µg/ml. The red coloration of red mud is due to high iron (Fe3+) levels in the primary ore minerals. The ability of A. Tubingensisto grow in the presence of different Fe concentrations was tested and the results indicated that the best growth of A. Tubingensis was achieved at concentrations of 400µg/ml. At higher Fe concentrations (500 and 600µg/ml) the growth was slightly increased when compared to control. There was a pH reduction of more than two units in 25% (9.3 to 7.1) and 50% (9.8 to 7.1) and almost three units in 75% (10 to 7.1) of red mud amended soils when inoculated with A. tubingensis. The present study showed that A. tubingensis play an important role in reducing the alkalinity of the red mud and also promote the plant growth, though bauxite residue is a poor substrate for plant growth because of very high pH, salinity and sodicity.

Das and Dandapat (2011) showed bioleaching of red mud is a major waste in the aluminium industry obtained by alkaline treatment of bauxite. The waste was used as a media for fungal growth and maintained in the form of a solution. Red mud was added to the organic solution to prepare samples of different pulp density (i.e.20%, 40%, 60% and 80% w/v).

The pH for different pulp densities of red mud with the period of incubation was observed after treatment with the fungal rich organic media. The pH as a function of initial pH, concentration of red mud and incubation period was modelled using the neural networks. The pH was observed for 30 different combinations of parameters like initial pH, concentration of red mud in media and period of incubation. The pH of as a function of initial pH, concentration and period of incubation has been modelled using the artificial neural network technique. They took a pure culture of indigenous fungi (Aspergilus niger) for production of organic acids results in a progressive decrease in the pH of the media which can also be associated with the fungal growth. The breakdown of glucose by glycolysis reduces the glucose flux and as a result causes a shift from citrate to oxalate accumulation. The leaching ability of fungi is due to its acidolysis and complication phenomena.

## 2.3EXPERIMENTS ON GEOTECHNICAL PROPERTIES OF RED MUD

Studies pertaining to geotechnical characterization of red mud are limited and are presented as follows.

Miners (1973) observed that red mud consists of sand and silt size particles with clay size up to 20-30%, with complete absence of quartz minerals and classified coarse grained fraction as red sand and fine grained fraction as red mud. .

Vogt (1974) described in situ undrained shear strengths are typically very high compared to uncemented, clayey soils at equivalent liquidity indices. The sensitivities vary from 5 to 15 with very high friction angles (φ) of 38-420 are also found for red mud.

Parekh and Goldberger (1976) observed that red mud is highly alkaline and its mineral components are generally hematite, goethite, gibbsite, calcite, sodalite.

Somogyi and Gray (1977) described red mud is of highly alkaline, having 20-30% clay sized particles, with the majority of particles in the silt range. One-dimensional compression tests indicate values for Cc ranging from 0.27 to 0.39 permeability k from 2 to $20 \times 10^{-7}$ cm/s and Cv $= 3 – 50 \times 10^{3}$ $cm^2/s$.

Vick (1981) observed that red mud is of low plasticity with liquid limit (LL) of 45% and plasticity index (PI) of 10% with relatively high specific gravity (GS) of 2.8-3.3. Due to its lack of clay mineralogy, these wastes show many geotechnical properties similar to clayey tailings found in other mineral processing [e.g., mineral sands, gold, etc].

Li (1998) found that red mud is highly alkaline (pH = 11-13) waste material, whose mineral components includes hematite, goethite, gibbsite, calcite, sodality and complex silicates and some red mud have been found to have greater than 50% of the particles less than 2µm. The cation exchange capacities of red mud are comparable with kaolin or illite minerals.

Newson et al. (2006) carried out investigations on physiochemical and mechanical properties of red mud at a site in the United Kingdom. Based on a set of laboratory tests conducted on the red mud, the compression behaviour found to similar to clayey soils, but frictional behaviour closer to sandy soils. The red mud appears to be “structured” and has features consistent with sensitive, cemented clay soils. Chemical testing suggests that the agent causing the aggregation of particles is hydroxyl sodalite and that the bonds are reasonably strong and stable during compressive loading and can be broken down by subjecting the red mud to an acidic environment. Exposure of the red mud to acidic conditions causes dissolution of the hydroxyl sodalite and a loss of particle cementation. Hydration of the hydroxyl sodalite unit cells is significant, but does not affect the mechanical performance of the material. The shape, size, and electrically charged properties of the hydroxyl sodalite, goethite, and hematite in the red mud appear to be causing mechanical behaviour with features consistent with clay and sand, without the presence of either quartz or clay minerals.

Liu et al. (2006) observed that pH value of red mud decreases with increase in duration of storage time and Oxygen(O) accounted for about 40% with other major elements included Calcium(Ca), Iron(Fe), Silicon(Si), Aluminium(Al), Titanium(Ti), Sodium(Na), Carbon, Magnesium(Mg) and Potassium(K) . XRD analysis shows calcite, perovskite, illite, hematite and magnetite are present in red mud and the old red mud also contained some kassite and portlandite. In addition, there are about 20% of amorphous materials in all red mud.

Sundaram and Gupta (2010) have some in-situ investigations on red mud to be used as a foundation material and they have observed that red mud is highly alkaline (9.3-10.2) with liquid limit of 39-45 %, plastic limit of 27-29% and shrinkage limit of 19-22%. They also found that undrained shear strength is 0.4 to 1.4 kg/cm2, specific gravity is 2.85-2.97, cohesion is 0.1 to 0.2 kg/cm2 and angle of internal friction is 26-280.

Rout et al. (2012) have designed high embankments based on the geotechnical properties of red mud by doing laboratory experiments. They observed that the maximum dry density (MDD), specific gravity and angle of internal friction are very high compared to local soil.

They concluded that red mud can be used as an embankment material by covering it with local soil.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 INTRODUCTION

This chapter discusses about the materials used in the present study. The main materials characterized in the present study are red mud and diary waste; experimental methodologies followed for characterization of these materials are discussed. A brief introduction about the above materials and methodology is presented in the following section in this chapter.

## 3.2 MATERIALS

### 3.2.1. Red mud

The red mud used in the research study is collected from NALCO, Damanjodi, Koraput in state of Odisha, India. About 0.8 to 1.5 tons of red mud is coming out per ton of alumina produced. The red mud is discharged in a slurry formto red mud pond shown in Figure 3.1. The area of red mud pond is about 212hectares. Due to environmental contamination water sprinkling system to arrest dust is shown in Figure 3.2.

Figure 3.1 Red mud pond of NALCO at Damanjodi

Figure 3.2 sprinkling system of red mud pond to prevent dust effect at Damanjodi

#### 3.2.1.1 Collection of red mud sample

The red mud is collected from the red mud pond in anair dried state and characterised by different standard laboratory methods. 100gms. of red mud was kept in freezer for microbial count and isolation of pure culture.

### 3.2.2 Dairy waste

The dairy industry is the most polluting among all food industry due to large consumption of water. Different research was going on for the better utilisation of dairy waste over worldwide. The waste coming from dairy industry was shown in Figure 3.3.

#### 3.2.1.1 Collection of Dairy waste

The dairy waste known as whey is prepared by taking OMFED milk and boiling it in induction cooker by addition of few amount of vinegar in saucepan in laboratory shown in Figure 3.3.

Figure 3.3 Laboratory whey

## 3.3 METHODS

The research work contain of experimental procedure for bioremediation of red mud and characterization and comparison of red mud before and after bio-neutralisation. The experimental methods refers to neutralise the red mud by microorganisms characterise the bio-neutralised red mud in terms of chemical, mineralogical and geotechnical properties and compare it with the original red mud. The experimental methods adopted in the present study are elaborated as follows.

### 3.3.1 Bioremediation methods

Bioremediation of red mud refers to neutralise the red mud by microorganisms. It contain isolation of microorganisms from red mud for getting pure culture from it, collect pellets from centrifuge method, neutralise red mud by using dairy waste. As the procedure are not familiar to geotechnical engineers so described separately below.

#### 3.3.1.1Isolation of microorganisms

Calculation of bacteria and fungi refers to standard plate count method. The soil sample was taken and diluted in different dilution factor as shown in Figure 3.4. The liquefied MRS agar medium was prepared and poured in to petri plates for solidification. The MRS agar and its preparation are described in the next section. Each diluted sample was spread in to the solid medium by L-shaped bent loop shown in Figure 3.5 known as spreading plate method. The levelled petri plates are incubated at $37^0$c for 48 hrs in B.O.D incubator for growth of microorganisms.

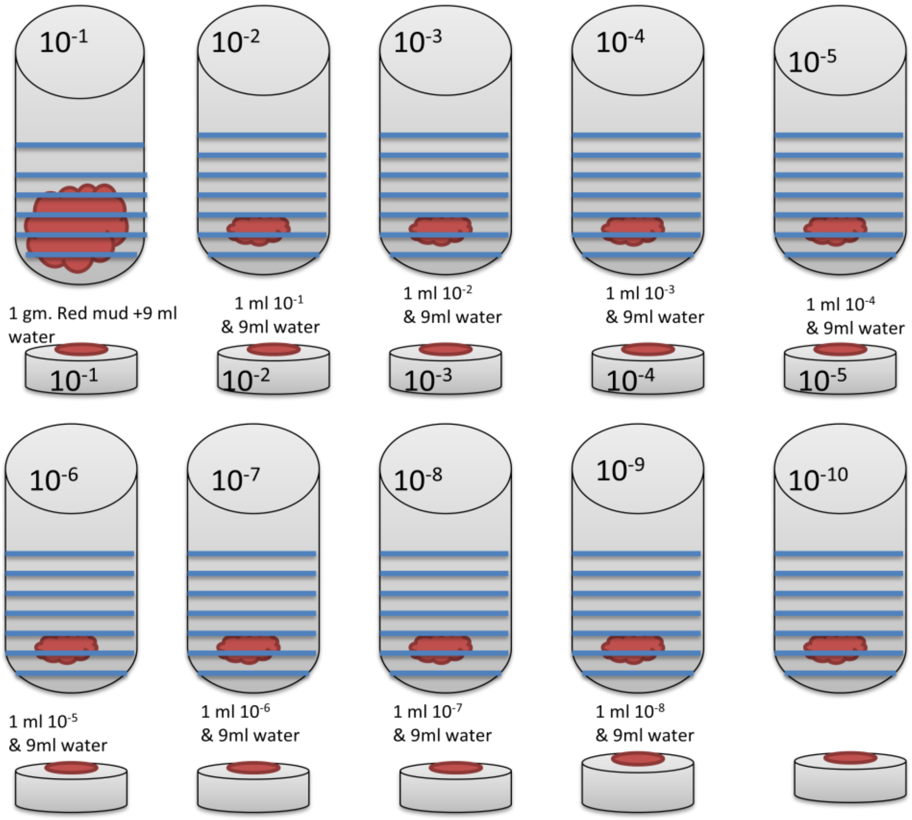


Figure 3.4 Serial dilution methods

Figure 3.5 L Shaped bent loop

### 3.3.1.2 Pure culture

Streak plate method is used for obtaining pure culture from colonies formed in petri plates. The streak plate method refers to four-quadrant streak pattern which used an inoculation loop for streaking. All strokes should be done in same direction not in both back and forth as shown in Figure3.6. The inoculation loop was heated up to red mud for sterilisation. After cooling the bacterial culture was spread to a large surface area of MRS agar for dilution process. The nichrome wire of loop are flamed to burn up any organisms and stroked through first quadrant when cooled.

Microorganism culture was taken to the second quadrant in the same procedure. The loop is heated after the second sector and the third quadrant is streaked like this the last fourth one.

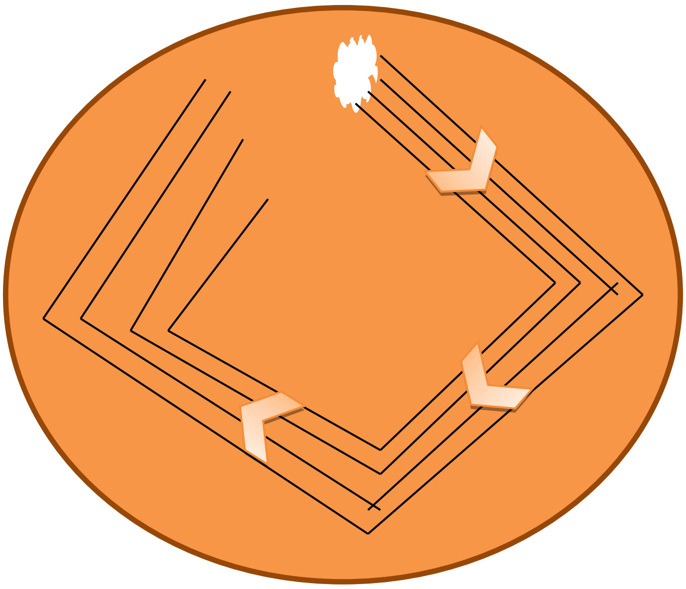

Figure 3.6 Streak plate methods

### 3.3.1.3 MRS agar

The media is so-named by its inventors: de Man, Rogosaand Sharpe. MRS agar typically contains(w/v)- 1.0 % peptone, egg extract of 0.8 %, 0.4 % yeast extract, 2.0 % glucose,0.5 % sodium acetate trihydrate, 0.1 % polysorbate, 0.2 % dipotassium hydrogen phosphate, 0.2 % triammonium citrate, 0.02 % magnesium sulfate heptahydrate, 0.005 % manganese sulfate tetrahydrate, 1.0 % agar, and pH adjusted to 6.2 at 25°C. Preparation of agar shown in Figure 3.8 is done by taking 67.15 grams in 1000 ml distilled water heated to boiling to dissolve the medium completely, autoclaved shown in Figure 3.7 at 15 lbs pressure (121°C) for 20 minutes. This media is designed to abundant growth of lactose fermenting bacteria. No pathogenic bacteria can survive in this specialised media

Figure 3.7 Autoclave

Figure 3.8 MRS agar preparations

### 3.3.1.4 Bioremediation of red mud

**Nursery trials**

The lactose fermenting pure culture bacteria was inoculated in two samples of lactose broth by an inoculation loop shown in Figure3.9 in sterilised laminar air flow. The pH was maintained to 11.04 by adding sodium hydroxide. It was kept in incubator at $37^0$c and pH was measured after 24, 48, 96 hrs.

The lactose broth is a liquid media for bacteria culture. The main composition of lactose broth is Peptic digest of animal tissue 5.000gms / Litre, Beef extract 3.000gms / Litre and Lactose 5.000gms / Litre and Final pH is maintained 6.9±0.2. The media is prepared by taking 13gms of lactose broth in 1000ml distilled water and autoclaved it at 15 lbs pressure (121°C) for 20 minutes.

Figure 3.9 Inoculation loop

### 3.3.1.5 Bio-neutralisation method

The quantity of bacterial culture is increased by centrifuge method which is described in section**3.3.1.6.** The pellet collected from centrifuge method was kept safely in refrigerator. Five different 100ml sample was prepared for the pH measurement. Two samples were prepared by taking 50 ml of water and 50gms of red mud. The other two samples were prepared by taking 50gms Red mud and organic solution of diary waste prepared in lab by boiled milk. The last sample was prepared by taking 50ml of lactose broth and 50 gm. red mud. Samples were sterilized and autoclaved at 121°C and 15lbs pressure to prevent microorganism growth other than our required species. The flasks were incubated on a shaker at 150rpm for 5 days at 370c in incubator. The pH of the red mud in the solution was monitored using a standard pH meter shown in figure 3.11. A large quantity of red mud was neutralised for the characterisation of geotechnical properties.

Figure 3.10 five different red mud samples

Figure 3.11 pH meters

### 3.3.1.6 Centrifuge

Centrifuge equipment is used to get pellets of bacterial culture from lactose broth. The equipment is driven by an electric motor, which makes rotate the object around a fixed axis. The principle is based on the sedimentation which causes separation of denser particles along the radial direction hence the pellet saved in bottom of the tube used. By this machine the supernatant and pellet can be separate out.

This machine should be carefully handled and balanced in the laboratory. The weights given in different tubes should be some which prevents force imbalance in centrifuge. Force imbalance can cause damage to the machine or personal injury when rotor is at high speed.

It should not be touched while rotation. Centrifuge normally identifies the amount of acceleration given to the sample; rather specify a rotational speed that is revolution per minute. The acceleration is often taken in multiples of g, refers to the acceleration of gravity at the Earth's surface. The acceleration is calculated by the product of the radius and the square of the angular velocity and radius. Due to the centrifugal force the pellets get separated in the bottom of falcon tubes, further it is taken care in laminar air flow. The centrifuge used is shown in Figure3.12.

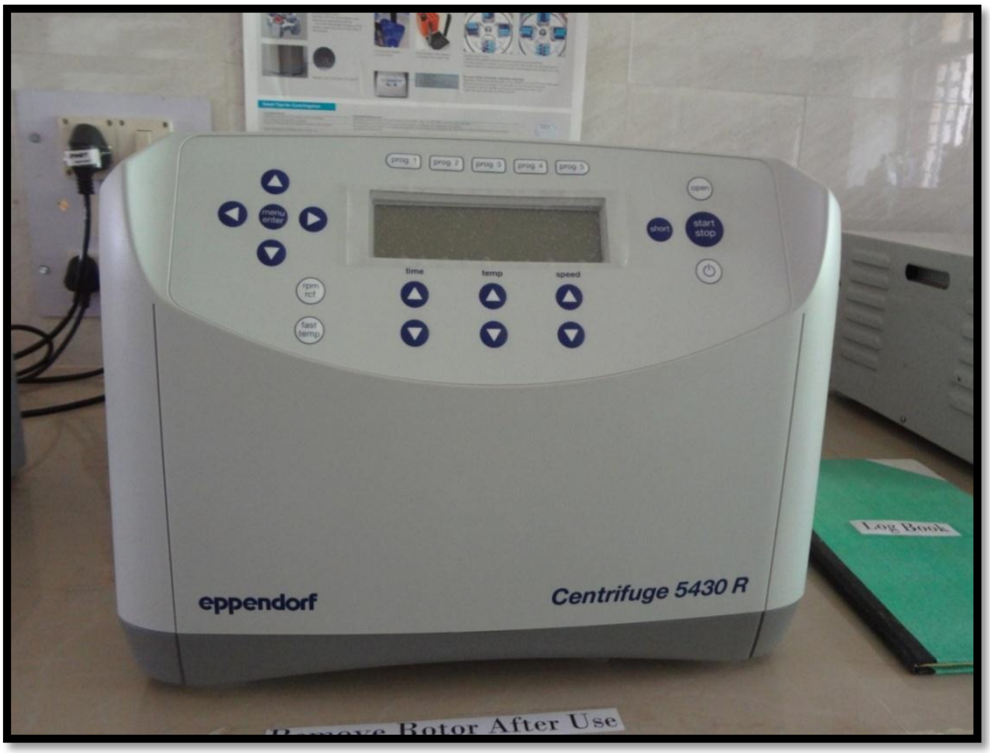


Figure 3.12 Centrifuge- 5430R

### 3.3.1.7 Staining procedure

Staining procedure is used to study the morphology of microorganism culture. Staining techniques used to improve contrast in the microscopic image in laboratory. Stains and dyes are frequently used in microbiology for highlighting structures in bacterial culture for viewing, often with the help of different microscopes. The procedure for staining of the isolated bacterial culture was described in this section.

The dried and cleaned micro slide was flamed for spreading the smear. The inoculation loop is flamed for red hot. A loop full of autoclaved water is transferred to the flamed slide surface. The entire nichorme wire is heated to red hot for sterilisation. The cap of tube is removed for holding the loop. The mouth of tube is flamed. The inoculation loop is touched to the tube to make sure it is cooled.

A pin size sample is picked up by loop without digging to the agar plate. The culture is dispersed on the slide in the drop of water and it is spread over an area in micro slide. The smear is allowed to dry. Heat-fix the smear is heat-fixed cautiously in the slide through the burner flame two or three times. The smear is stained by Methylene blue for 1 minute, Crystal violet for 30 second. The slide is rinsed off with tape water to wash out the excess stain. The back of slide is wiped by paper towel. The stained smear is placed on the microscope stage and is focused the smear using the 10X objective. The smear can be focused with the 100X objective by applying oil. The bacteria culture can be shown in the microscope shown in Figure 3.13.

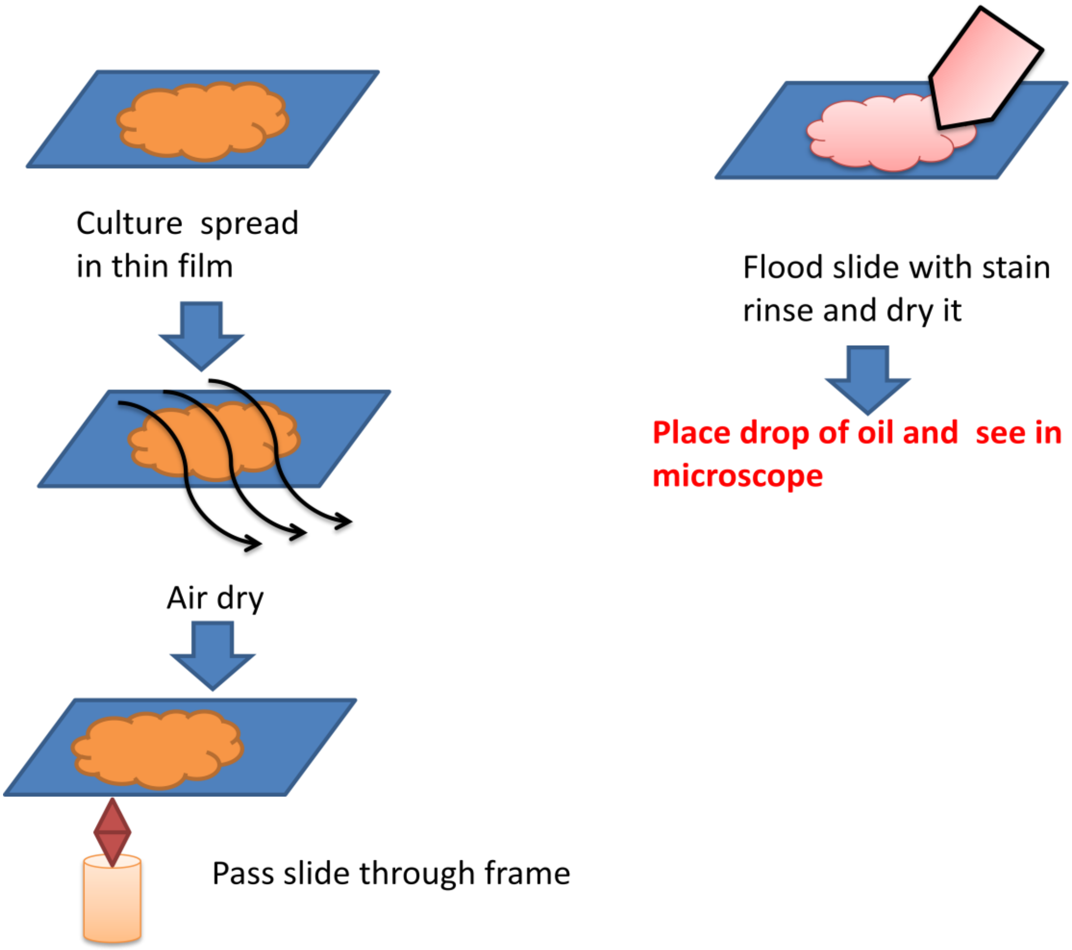


Figure 3.13 Staining Method

### 3.3.2 Characterisation of Red mud

#### 3.3.2.1 X-Ray Diffraction Analysis

X-Ray Diffraction (XRD) technique is used for the mineral phase's characterisation for the collected red mud. X-ray diffraction method refers Rietveld refinement methods which used to take out on the collected red mud for useful classification of the mineral phases and quantitative approximation of mineralogical composition. The powdered form of red mud samples were dried up at 105°C for X-ray diffraction analysis. First the red mud powder is taken for mineral phase identification.

The powdered sample is characterised by passing all the way through a Philips diffract meter with a Cu Kα radiation source and a single crystal graphite monochromator. The angular range is given at 10–70° of 2θ value in 0.1° increments throughout. The XRD instrument used in the present study is shown in Figure 3.14.

Figure 3.14 X-Ray Diffract meter Instrument

### 3.3.1.8.2 Study of the Geotechnical Properties

Several physical and chemical properties that are of particular concern on red mud samples as a construction material for fill and embankment are particle size distribution, specific gravity, atterburg's limit and standard compaction test, unconfined compressive test, direct shear test etc. Each and every geotechnical properties of red mud have been characterised as per IS2720 and part 1 of SP 36. The pH values of all the samples found out by Electrometric pH meter and rechecked by pH paper conducted as per SP 36. The different geotechnical instruments are shown below in Figures3.15.


Figure 3.15 Unconfined compression test

### 3.3.1.9 Characterisation of dairy waste

### 3.3.1.9.1 Introduction

Whey or dairy waste is the remaining liquid coming out from milk when cheese is prepared or during removal of fat or proteins from milk. Whey is a colloidal solution of small amount of fatty acid of bacterial solution after removing it; whey contains proteins, carbohydrate (lactose) and minerals with crystal clear yellowish colour.

#### 3.3.1.9.2 Total hydrocarbon content

The anthrone reaction procedure is the most suitable and reliable method to calculate total carbohydrate contain in free or in any solution. Due to the use of sulphuric acid Carbohydrates get hydrolysed in simple sugars. Glucose gets dehydrated to hydroxyl methyl furfuralin the environment of hot acidic medium. The solution forms a green coloured solution with anthrone with an absorption maximum at 630 nm. The procedure is described below. About 16 ml of diluted sulphuric acid is put in freezer before 4 hrs of experiment. 0.2% (32mg) of anthrone is added in the closed container and mixed it carefully. Three cleaned test tubes is used as one for blank solution, one for standardised solution and last one for estimation of total carbohydrate of the sample. 0.5ml of distilled water is kept in last two solution and 0.5ml of standardised and sample for estimation respectively. It is kept in water bath at $100^0$c for 10 minutes. The test tubes are kept for cooling and then used to determine the carbohydrate contain in the sample. The estimation can be done using the formula given below or with the standardised curve. The test tubes for blank and dairy waste sample, which produce green colour by reaction in shown in Figure 3.18.

$$\frac{Absorbance\ of\ unknown}{Concentration\ of\ unknown} = \frac{Absorbance\ of\ standard}{Concentration\ of\ standard}$$

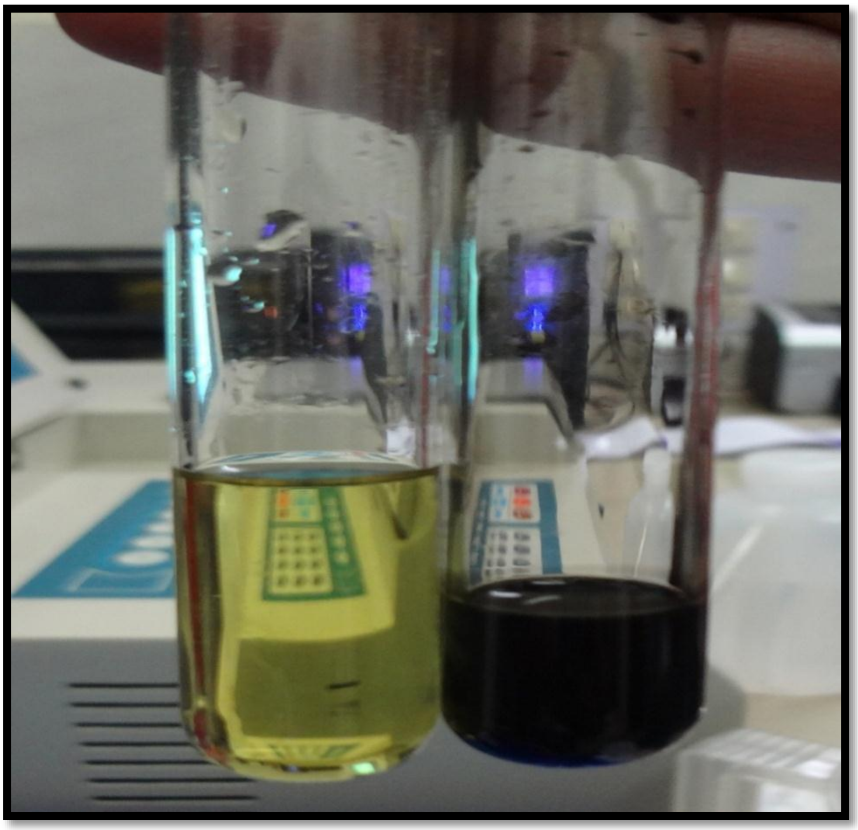

Figure 3.16 Test tubes for blank and dairy waste sample react with anthrone

### 3.3.1.9.2 Spectrophotometer

The instrument is shown in Figure 3.17. This is an instrument for measuring the quantity of light of a particular wavelength which passes throughout a medium. The instrument is based on the principle of Beer's law which states that the amount of light absorbed by a medium is proportional to the concentration of the absorbing solution used. Hence concentration of a coloured solution may be determined by measure the absorbency of light at a given wavelength in the laboratory. The solution is marked as B for blank and S for sample as per convince. The cuvette should be used carefully as it is expensive and basic need of the instrument.

The cuvette should not be scratched and clean after every use so that no disturbance should occur when light of 620 nm is passed from the solution.

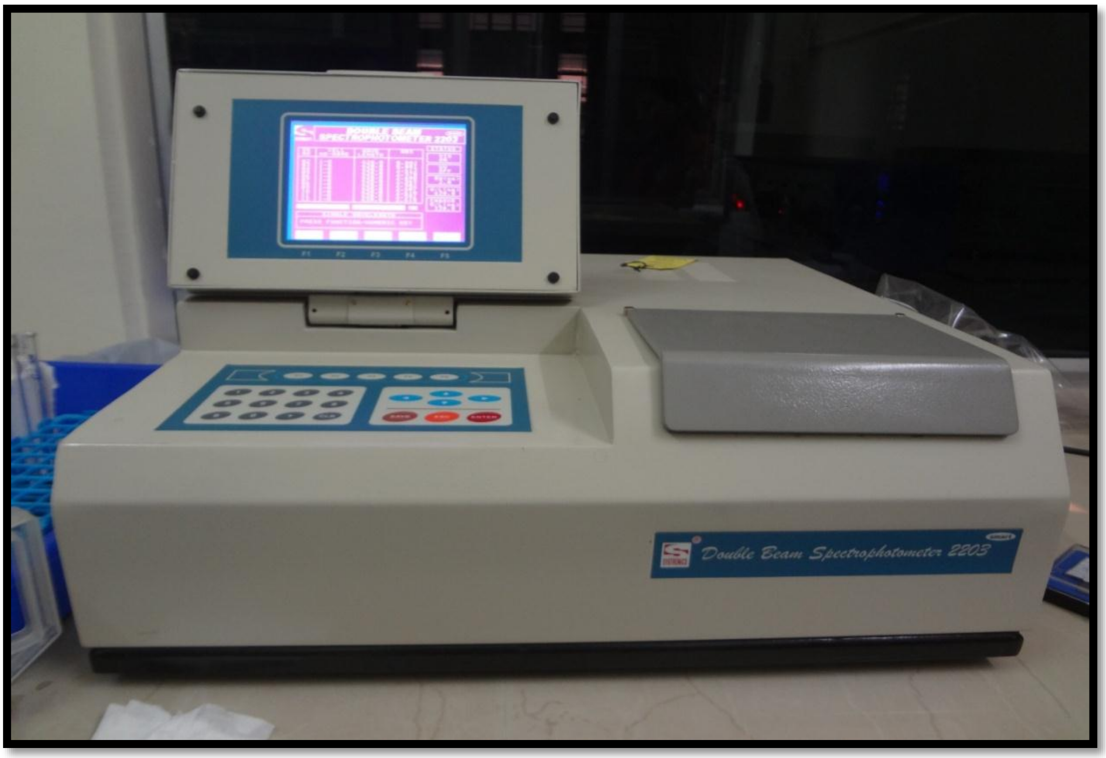


Figure 3.17 Spectrophotometer

### 3.3.1.9.3 Atomic adsorption test

Atomic absorption spectroscopy is used to conclude the quantitative chemical elements using of the sample. The fundamental ideology of atomic absorption is the electrons of the atoms is promoted to higher orbital in the atomizer for a few  time by absorption of a distinct amount of energy which come by radiation of wave length. The quantity of energy is particular in a particular element to a distinct electron transition. This principle based on Beer-Lambert Law hence the chemicals are measured by detector of the sample. The instrument used is shown in Figure 3.18.

Figure3.18 Atomic absorption spectrophotometer

## CHAPTER 4

## BIOREMEDIATION AND CHARACTERISATION

### 4.1 BIOREMEDIATIONOF RED MUD

#### 4.1.1 Introduction

The experiment for isolation, Bio-neutralisation is done as per discussed in chapter 3. The results coming out are mentioned briefly in this chapter with figures. The bio-neutralised red mud is further used for geotechnical characterisation of red mud.

#### 4.1.2 Isolation of bacterial culture

Ten different levelled petri plates having agar and different dilution of samples are incubated for the growth of lactose fermenting microorganism. The result shows four different colonies are formed in the petri plates after 48 hrs. The colonies formed are used for obtaining pure culture which is shown in Figure 4.1.

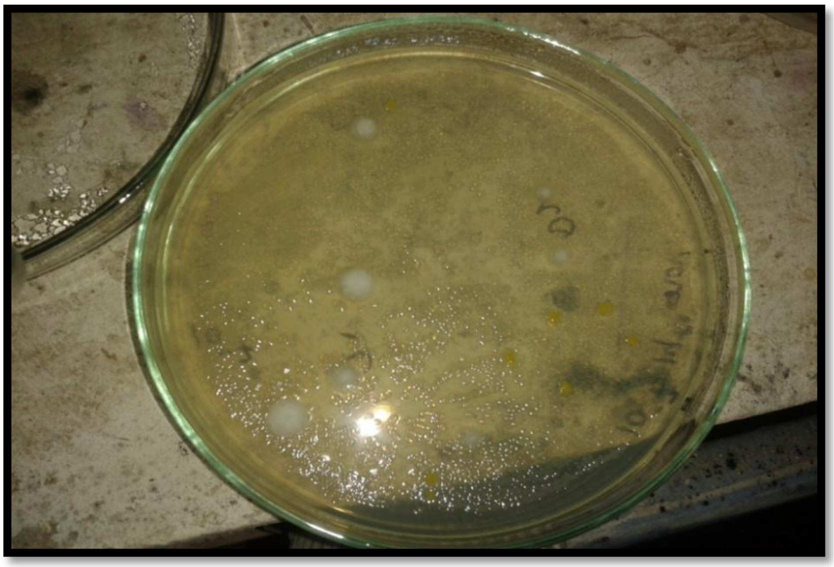

Figure 4.1 colonies formed in petri plate

### 4.1.2 Pure culture

As Streak plate method, the most reliable method is used for the pure culture. The four colonies were stroke for pure culture and incubated in incubator. Result shows colonies are absolutely pure and no need to do more striking. The culture is saved in agar form in freezer. The culture is used for nursery trials. The pure culture is shown in Figure 4.2.

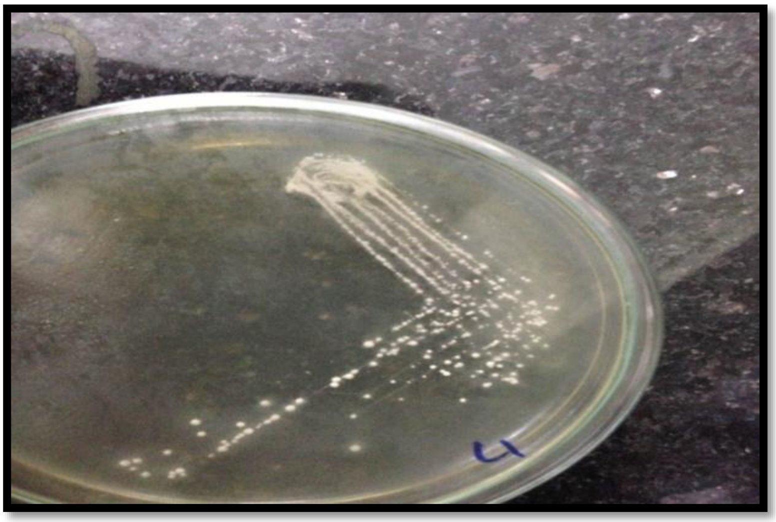

Figure 4.2 Pure culture obtained from red mud

### 4.1.3 Morphology study

The pure culture obtained is studied with respect to their structural form. Morphological study is done of the bacterial culture by staining procedure as described in section**3.3.1.7.** The result shows the four pure cultures are same in structure which is used for bio-neutralisation. The bacterial structure in 100x microscope is shown in Figure 4.3.

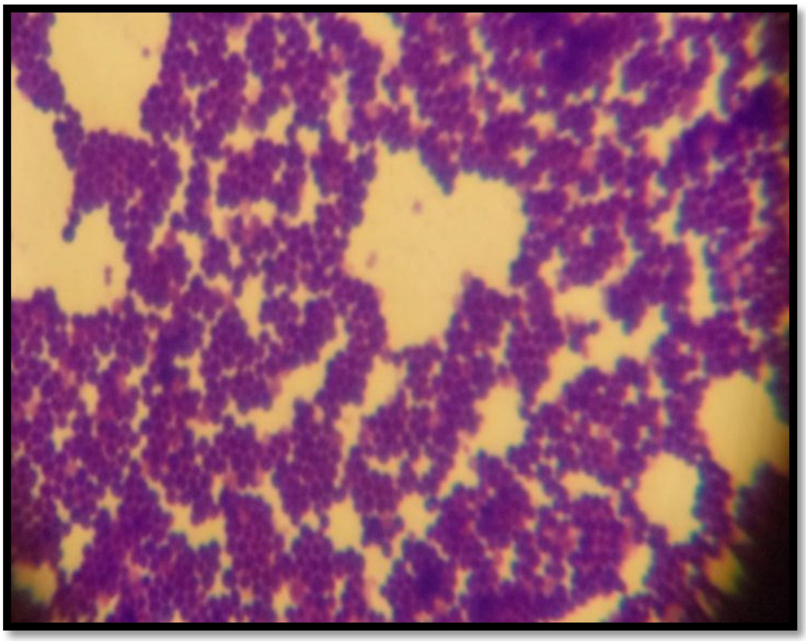

Figure 4.3Bacterial cultures in microscope

The morphological study shows in above figure isCoccusin shape. The colour of bacteria culture is off white in colour which converts purple when bacterial cell absorbed the dye, which helps in staining process.

**4.1.4 Nursery trials**

The bacterial culture is tested for reduction of pH of lactose broth in nursery trials. The pH is shown in Table 4.1, which was monitored while, incubation.

Table 4.1 pH monitoring of lactose broth

| Time of incubation | 1st sample (lactose broth + bacteria) | 2nd sample (lactose broth + bacteria) |
|---|---|---|
| 0 hour | 11.04 | 7.11 |
| 24 hour | 8.82 | 7.11 |
| 48 hour | 8.43 | 4.74 |
| 96 hour | 5.59 | 4.56 |
| After 7 days of incubation | 5.56 | 4.77 |

The result shows that the obtained bacterial culture lactose fermenting facultative bacteria which can convert lactose to lactic acid in their metabolism process. The bacterial culture can live in the high alkaline environment as well as in the acidic environment.

**4.1.5 Bio-neutralisation process**

In bioremediation there is no change of pH value of red mud and inoculated cultured with red mud. There is a reduction of pH of red mud with lactose broth and inoculated culture but it's not feasible. The pH value reduced to 8.40 and 7.40 with diluted and non-diluted diary waster when mixed with red mud with the bacteria culture. The variation of pH was shown in table 4.2 and in Figure 4.4.

Table 4.2 pH measurement of red mud with different solution

| Days of incubation | Red mud & water | Red mud, Water & bacteria | Red mud, Lactose broth & bacteria | Red mud, dil. Diary waste & bacteria | Red mud, diary waste & bacteria |
|---|---|---|---|---|---|
| 0 days | 10.06 | 10.04 | 10.04 | 9.60 | 9.60 |
| 2 days | 10.06 | 9.90 | 9.60 | 8.67 | 7.82 |
| 4 days | 10.06 | 9.90 | 9.47 | 8.40 | 7.40 |
| 6 days | 10.06 | 9.90 | 9.23 | 8.40 | 7.40 |
| 8 days | 10.06 | 9.90 | 9.23 | 8.40 | 7.40 |

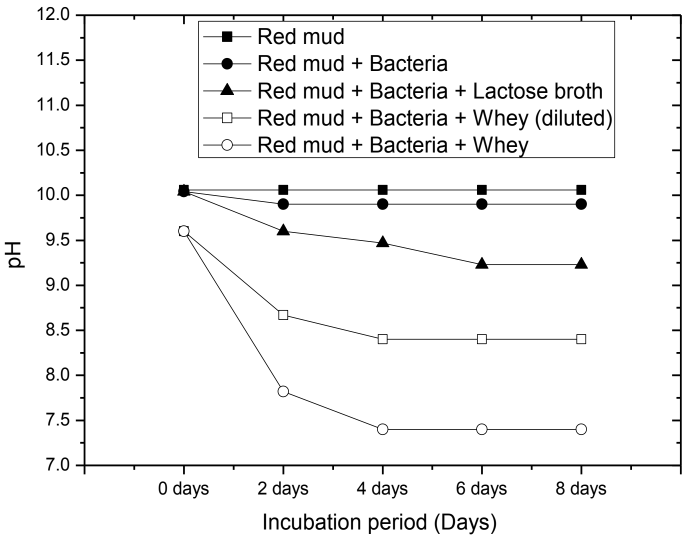


Figure4.4 Variation of pH value of red mud under different conditions

### 4.2 CHARACTERISATION OF DAIRY WASTE

#### 4.2.1 INTRODUCTION

Crystal clear yellowish colored whey contains carbohydrate, protein and different minerals. The carbohydrate contains lactose and is calculated in anthrone method. The minerals are calculated in atomic adsorption machine. The content are presented in the below table 4.3.

Table 4.3 contents of dairy waste

| Sl. No | Contents | Amount |
|---|---|---|
| 1 | Lactose | 47g/lit |
| 2 | Fe ( Iron) | 0.455mg/l |
| 3 | Chloride | 650mg/l |
| 4 | K (Potassium) | 48.947mg/l |
| 5 | Mg(Magnesium) | 6.604mg/l |
| 6 | Ca(Calcium) | 61.865mg/l |
| 7 | Na( Sodium) | 13.700mg/l |

### 4.3 RED MUD CHARACTERISATION AND COMPARISON

#### 4.3.1 Introduction

The red mud and bio-neutralised red mud material properties is discussed and presented in this section. The procedure for characterisation is already described in pervious chapters. The mineralogical and all geotechnical properties are presented and compared with bio-neutralised red mud. Hence it can be known that the bio remediated red mud can be used for construction material as fill and an embankment or not.

### 4.3.2 Mineralogical study

The X-Ray Diffraction analysis of red mud is shown in Figure 4.5 and from the analysis it is observed that Hematite, Boehmite, Gibbsite, Rutile, Goethite and Sodalite are major minerals present in red mud. The bio-neutralised red mud X-Ray Diffraction analysis is shown in Figure 4.6 and it was observed that similar types of minerals are observed after bioremediation qualitatively. However, some quantitative differences were observed, though it has not been measured.

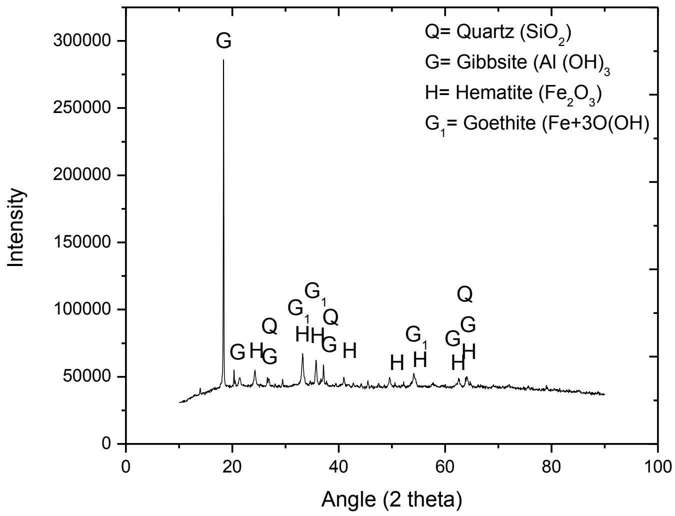


Figure 4.5 XRD analysis of red mud

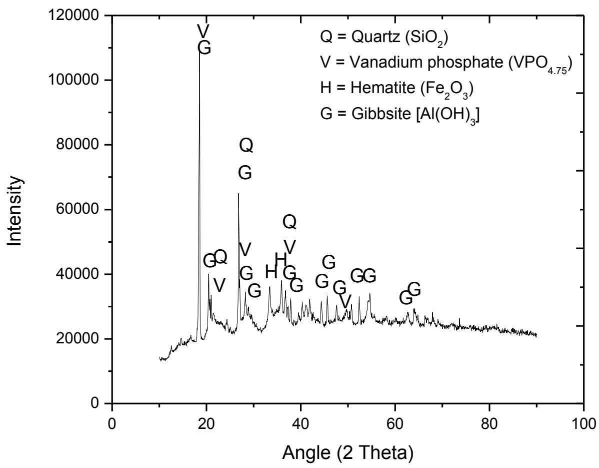


Figure 4.6 XRD analysis of bio-neutralised red mud

### 4.3.3 Geotechnical Properties

The colour of the red mud is normally brown to reddish brown due to the presence of iron oxide. There is no distinct colour change in red mud after neutralisation. The basic geotechnical characterisation are compared and presented below in the table.

#### 4.3.3.1 Specific Gravity

Specific gravity test was done for both the red mud and bio-neutralised red mud. The specific gravity of red mud varies between 2.8 to 3.3 as per the literature review. Particle heterogeneity is the main cause of variation of red mud.The specific gravity is reduced from 3.34 to 2.83 when it is bio-neutralised. The main cause of reduction of specific gravity is due to the presence of organic matter as a lactic acid.

#### 4.3.3.2 Atterburg limits

Liquid limit (LL) and Plasticity index (PI) of red mud and bio-neutralised red mud are evaluated. As the LL and PI of red mud are found to be 24.75% and 7.25%, respectively, red mud is found to be Inorganic silts of low plasticity (ML), and inorganic clays of low plasticity (CL) (ML-CL) as per Indian classification. The bio-neutralised red mud liquid limit and plasticity are 23.34%, 3.48% respectively. The liquid limit and plasticity index is also reduced when it got neutralised by using dairy wastes.

#### 4.3.3.3 Compaction Characteristics

The standard Proctor (light compaction) test result for red mud is presented here. The maximum dry density (MDD) of red mud is found to be 19.8kN/m3 at light compaction with 18% of optimum moisture content. It may be mentioned here that the Gs value of red mud vary from 2.99 – 3.43. The MDD and OMC are used for unconfined compressive strength of red mud.

#### 4.3.3.4 Unconfined Compressive Strength (UCS)

Keeping in mind, use of red mud as an embankment material, the shear strength of the red was obtained through unconfined compressive strength.

Table 4.4 Geotechnical properties of red mud and bio neutralised red mud

| Sl. No | Properties | Red mud | Neutralised red mud |
|---|---|---|---|
| 1 | pH value | 10.06 | 7.40 |
| 2 | Specific gravity | 3.34 | 2.83 |
| 3 | Liquid Limit | 24.75 | 23.34 |
| 4 | Plastic Limit | 17.5 | 19.86 |
| 5 | Plasticity Index | 7.25 | 3.48 |
| 6 | Unconfined Compressive Strength | 57.6 | 41.11 |

# CHAPTER 5

# CONCLUSION AND FUTURE SCOPE

## 5.1 SUMMARY

About 75 million tons of red mud is produced per year in aluminium industry over worldwide. The red mud should be used in a huge quantity for the construction material as embankments and fills without any environmental hazard. So the red mud is neutralised and it can be used in terms of soil in construction material. The present study concludes the laboratory tests like morphology, chemistry and geotechnical index properties of red mud and bio-neutralised red mud. The red mud is neutralised by using dairy waste and lactose fermenting bacteria. A comparison of some properties has been made of red mud and bio-neutralised red mud.

## 5.2 CONCLUSIONS

The following observations can be made based on the above from Chapter 1 to Chapter 4. Production of red mud is an alarming environmental effect and for storage it needs a vast land area. The red mud production is 1.2 – 1.4 times the production of alumina which is a big issue for the world. Though, several labours have been made to use red mud an alternative material in building and alloy industries, but only 5% of red mud can be used.

1. The toxicity of red mud is the main cause for which it cannot be used. The toxicity is due to the high alkaline in nature so in this research the red mud is neutralised by microbes so that it can be used in construction material as well as lessen the environmental effect and cost for storage of red mud.

2. There is a potential to use in huge quantities as a fill and embankment material, but very little efforts have been made to neutralise and characterize neutralised red mud as a geotechnical engineering material, particularly the Indian red mud.

3. The alkalinity of red mud is high with a pH value 10.06 due to presence of NaOH and $Na_2CO_3$, these are expressed in terms of $Na_2O$.

4. The isolated bacteria culture can reduce the pH value of red mud to 7.40 from 10.06 when red mud is mixed with diary waste product.

5. The specific gravity changed to 2.83 from 3.34 due to the presence of organic matter.

6. There is a variation of plasticity index due to slight decrease in liquid limit and increase in plastic limit.

7. The unconfined compressive strength value is decreasing to 41.11 kPa from 57.6 kPa at optimum moisture content.

8. High specific gravity of red mud (2.99-3.43) may be attributed to mineral content like Hematite. The plasticity is low and can be classified as ML-CL. The specific gravity and plasticity got reduced when red mud is neutralised.

9. There is a reduction of plasticity index and cohesion due to the loss of apparent cohesion present in red mud.

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## 5.3 FUTURE SCOPE

There is a vast scope to use red mud as fill and embankment material in huge quantities. The neutralisation and geotechnical characterization of red mud in this study is limited to a single source and laboratory investigations. Some of the followings are recognized for future studies.

(1) In-situ studies and its laboratory validation of properties of red mud from different sources and different storage times. (2) Application of this procedure in field to neutralise the red mud and its efficiency (3) Stabilization of neutralised red mud using other industrial waste and local soil (4) The effect of temperature and humidity of the field in micro organism(5) Effect of other microorganisms present in red mud (6) Model footing on foundations, slopes and retaining walls under static and dynamic load using advanced instrumentation, centrifuge modelling and numerical validation of models using FE analysis. (7) Importance of microorganism and bio mass in geotechnical engineering.

**REFERENCE**


1. Mussels G., Sparkling G., Summers J (1993). ‘‘Bioremediation of bauxite residue in Western Australia.’’-An initial feasibility study, No. 26. Alcoa of Australia ISSN 1320-4807.

2. Hamdy M.K. and Williams F.S. (2001). ‘‘Bacterial amelioration of bauxite residue waste of industrial alumina plants.’’, *J. Ind. Microbiol. Biotech.* No.**27**, 228–233.

3. Krishna P., Reddy M. and PatanikS.K. (2005). ‘‘Aspergillus tubingensis reduces the pH ofthe bauxite residue (red mud) amended soils.”,*Water, Air, and Soil Pollution* No. 167, 201–209.

4. Diamond D. and DandapatSnigdha J. (2011). “Artificial Neural Network Modelling for the Study of pH on the Fungal Treatment of Red mud.”,*Res.J.Chem.Sci.* ,No.1, 108-112.

5. Vogt, M. F. (1974). “Development studies on dewatering of red mud.” 103rd Annual Meeting of AIME, Dallas, Tex., 73-91.

6. Somogyi F. and Gray D. (1977). “Engineering properties affecting disposal of red mud.” Proc., Conf. on Geotechnical Practice for Disposal of Solid Waste Materials, ACSE, 1-22.

7. Rout S., Sahoo T. and Das S.K. (2012). "Utility of Red Mud as an Embankment Material." *Inter National Journal of Earth Sciences and Engineering*. No.06, 1645-1651.

8. Rai s., WasewarK.L., Mukhopadhy J., YooC.K. and Uslu H. (2012). "Neutralization and utilization of red mud for its better waste management" *Arch. Environ. Sci*. no.06, 13-33.

9. Li, L. Y., Properties of red mud tailings produced under varying process conditions, J. Environ. Engg. 1998, Vol-124 (3), pp.254-264.

10. Liu, W.; Yang, J. & Xiao, B. (2009), Application of Bayer red mud for iron recovery and building material production from aluminosilicate residues, Journal of Hazardous Materials, Vol.161, pp.474-478. ISSN 03043894.

11. Miners, K., Alcan's experience of the disposal of red mud from the Bayer ore process, Proc., 1st Int. Tailings Symposium, Miller Freeman, San Francisco, 1973, 553-562.

12. Newson, T., Dyer, T., Adam, C. and Sharp, S., Effect of structure on the geotechnical properties of bauxite residue, J. Geotech. & Geoenviron. Engg., 2006, Vol-132:2, pp.143- 151.

13. Parekh, B., and Goldberger, W., An assessment of technology for possible utilization of Bayer process muds, USEPA, 1976, EPA-600/2-76301.

14. Somogyi, F., and Gray, D., Engineering properties affecting disposal of red mud, Proc., Conf. on Geotech., Practice for Disposal of Solid Waste Materials, ACSE, 1977, 1-22.

15. Sundaram, R. and Gupta, S.  Constructing Foundations on Red Mud, 6th International Congress on Environmental Geotechnics, 2010, New Delhi, India, pp. 1172-1175.

16. Vick, S. G., Planning, design and analysis of tailing dams, Wiley, New York, 1981, 369.

17. Vogt, M. F., Development studies on dewatering of red mud. 103rd Annual Meeting of AIME, Dallas, Tex., 1974, pp.73-91.

**PUBLICATIONS FROM THIS STUDY**

**Conference**

1. **Surabhi Jain,** S.K.Das, (2014). "Characterization Of Indian red mud as a fill and embankment material using Bioremediation", 7th International Congress on Environmental Geotechnics (ICEG), Malbourne, Austrilla
2. **Surabhi Jain,** S.K.Das, (2014). "Geotechnical characterisation of bio-modified red mud", Innovation in Science & Technology for inclusive Development, REGIONAL SCIENCE CONGRESS, January 27-28, 2014